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TITLE:

Defining High-Risk Precursor Signaling to Advance Breast Cancer Risk Assessment and Prevention

PRINCIPAL INVESTIGATOR:

Dr. Leif Ellisen

**CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, MA 02114**

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14. ABSTRACT Making a major impact on the incidence and lethality of breast cancer will require a detailed understanding of the earliest tissue changes that ultimately drive the process of breast cancer development. There is no substitute for the ability to define and understand the early, pre-malignant changes as they occur in women who are breast cancer-predisposed. One group of women at high breast cancer risk (up to 80% lifetime breast cancer risk) are those who have inherited mutations in the BRCA1 and BRCA2 genes. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. We have established an IRB-approved protocol that allows us to collect and analyze a portion of this tissue. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies are likely to lead directly to clinical trials of new approaches to prevent breast cancer.					
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Making a major impact on breast cancer will require a detailed understanding of the earliest tissue changes. One group of women at high breast cancer risk are those with BRCA1 and BRCA2 mutations. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies should lead directly to clinical trials of new approaches to prevent breast cancer.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Breast cancer; BRCA1/2; cancer prevention; paracrine signaling

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.

Major Tasks: Functional quantitation; Functional analysis; Signaling Analysis
(Completed in Months 1-12)

Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.

Major Tasks: Transcriptomics (completed month 12); genomics (in process); bioinformatic integration (in process).

Aim 3: Block abnormal signaling in vitro and in vivo

Major Tasks: Reverse abnormal signaling in vitro and in vivo (in process).

What was accomplished under these goals?

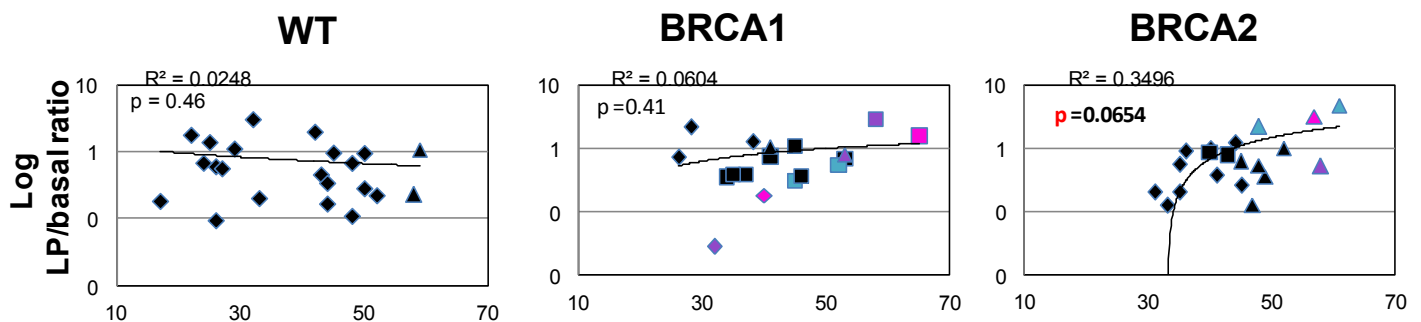
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

NB: We are pleased to share the remarkable accomplishments under this award to date. As specifically requested, accomplishments are now organized and enumerated based on tasks corresponding to the original SOW for this award. Accomplishments are supported by tables and figures as specifically requested.

Specific Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.

Major Task 1: Quantitation of LP (Luminal Progenitor) and basal stem cell (MASC) populations

A. Quantitation of LP and basal stem cell (MASC) populations



Methods and accomplishments (Figure 1 above):

Method: Primary mammary tissues from each patient underwent enzymatic digestion, followed by specific antibody labeling and FACS as described in detail in the original proposal.

Accomplishments: Shown is the ratio of LP to basal (MASC) population for each patient. We accomplished the novel and remarkable finding that there is a near significant age-associated increase in ratio of LP to basal cells specifically in BRCA2 carriers. This finding supports the hypothesis of a deregulation of the LP population in BRCA2 carriers.

Diamond= premenopausal, no HRT; Square= Post menopausal, +HRT

Triangle= Post menopausal, no HRT; Pink= breast cancer; neoadjuvant chemo

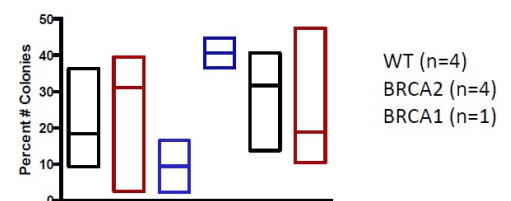
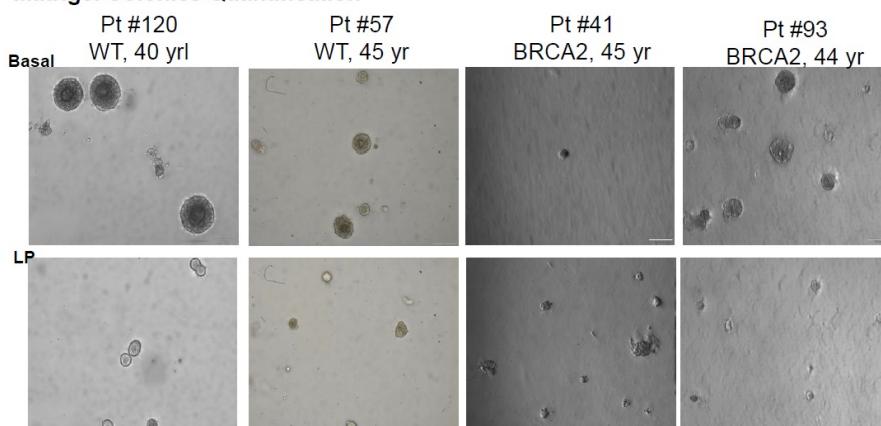
Purple= breast cancer; no neoadjuvant chemo; Teal= Ovarian cancer; prior chemo

B. CFU and mammosphere assays for functional quantitation

Methods and accomplishments: (Figure 2 below).

Method: Primary FACS-sorted mammary epithelial cells were plated in matrigel and percent of plated cells forming colonies was tabulated.

Matrigel Colonies Quantification



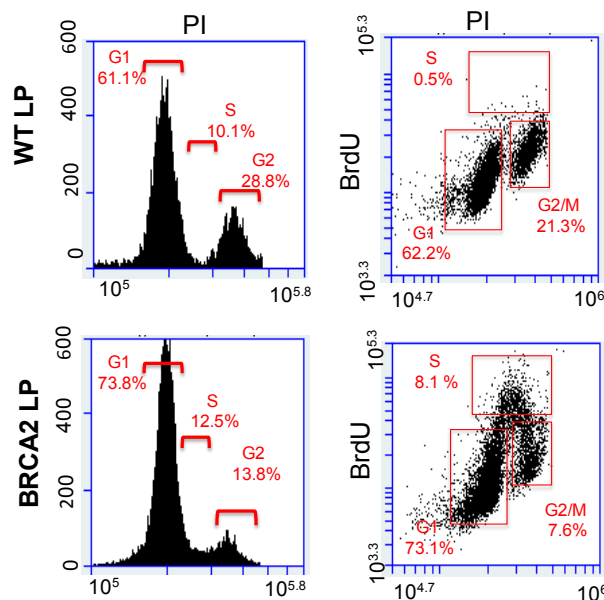
Accomplishments: Shown at left are representative matrigel assays: top, basal cells; bottom, LP cells. Shown at right is the summary: box shows range, and horizontal line shows mean; left boxes show basal cells, right boxes show LP cells; black: WT; blue: BRCA1; red: BRCA2. These results support the hypothesis of replication stress in BRCA2 LPs leading to more limited growth in matrigel.

Major Task 2: Functional analysis of LP and basal stem cell (MASC) populations

A. Proliferation (CFU) assays in presence and absence of growth factors.

Methods and accomplishments: (Figure 3 below).

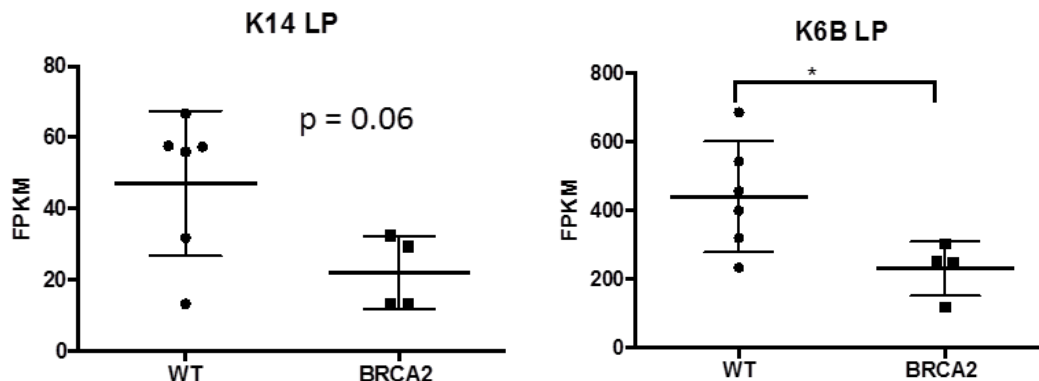
Methods: Primary FACS sorted mammary epithelial LP cells from WT and BRCA2 carrier patients were stained with propidium iodide (PI) (left graphs) or PI and BrdU (right graphs) and quantitated by FACS. Y-axis at left = cell number; Y-axis at right = BrdU intensity. X-axis = PI intensity in both graphs.



Accomplishments: These studies demonstrate for the first time a deregulated proliferation of primary FACS-sorted LP cells from a BRCA2 carrier. We hypothesize that this deregulated proliferation is reflected in the deregulated LP cell number shown in Figure 1.

B. Assessment of bi-lineage differentiation

Methods and accomplishments: (Figure 4 below).



Methods: RNA was extracted and sequenced from primary FACS-sorted mammary epithelia from non-carrier (WT) control patients and BRCA2 carriers. Genes related to cell fate and cell lineage were analyzed for alterations in the control versus BRCA2 populations. Graphs show gene expression (Y-axis, arbitrary values) of Keratin 14 (K14) and Keratin 6B (K6B), both basal differentiation markers, from non-carrier subjects (circles) and BRCA2 mutation carriers (squares). Each spot represents gene expression in one patient's LP sample. Vertical bars show standard deviation and middle horizontal bars show means. *P=0.06 for both comparisons.

Accomplishments: These experiments show for the first time decreased basal-like differentiation in LP cells from BRCA2 mutation carriers. These findings are consistent with the hypothesis that not only cell numbers but also cell fate is deregulated in the BRCA2 LP population.

Major Task 3: Analyze signaling in high-risk tissues in vivo.

IHC to analyze signaling in high risk tissues and controls in vivo
Methods and accomplishments: (Figures 5 and 6 below).

Methods: Fixed tissues from control patients and BRCA1/2 carriers were subjected to immunohistochemistry for the proteins detailed below. Figure 5 below shows Ki67 (proliferation marker). Clinical characteristics of each subject are indicated. Fractions refer to number of KI67 positive epithelial cells/total number of cells counted.

Ki67 Staining in Lobules of WT vs BRCA2 carriers

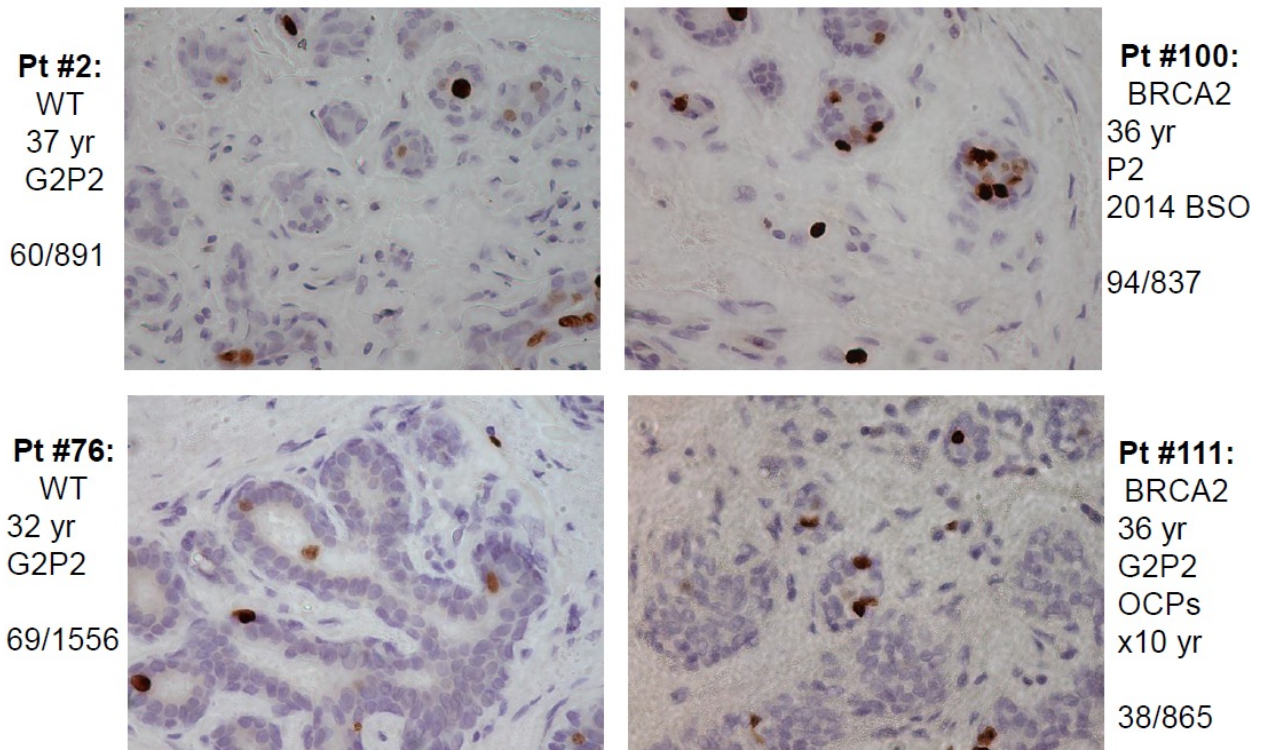
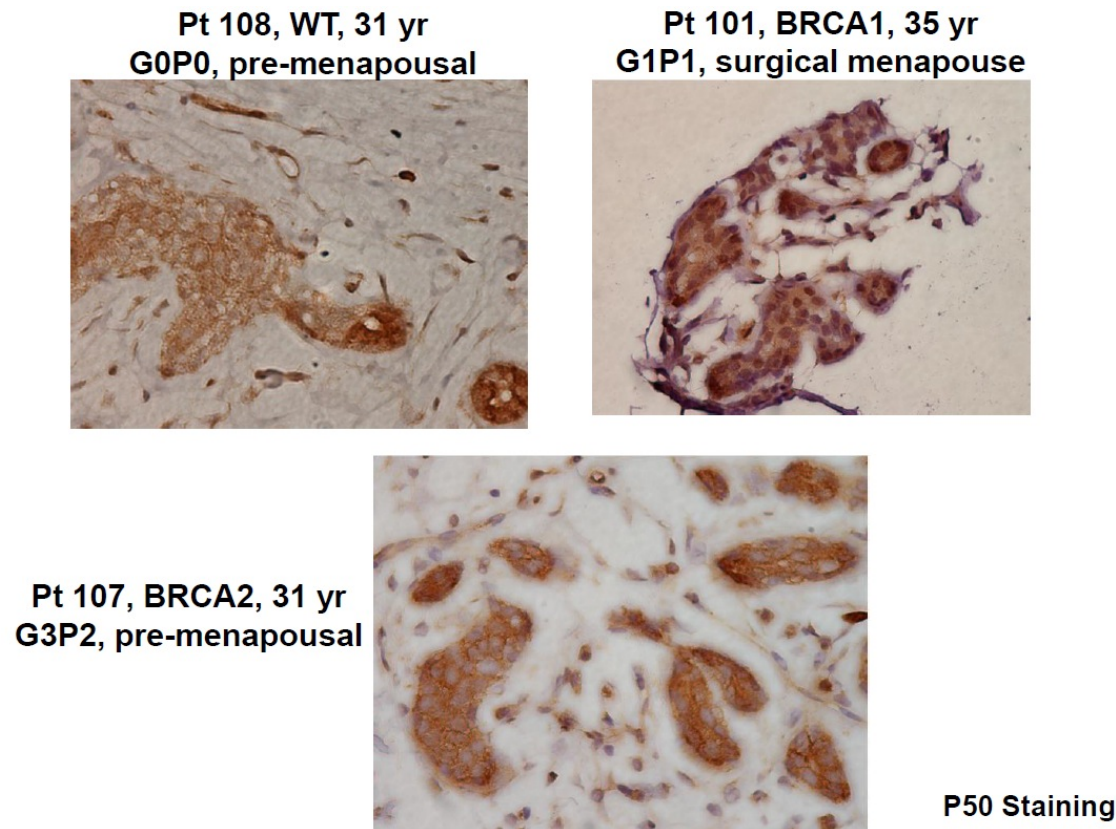


Figure 6 below shows representative staining for the NF-KB subunit p50 in WT, BRCA1 and BRCA2 carrier mammary epithelia in vivo. Clinical characteristics of each subject are indicated. Activated NF-KB is evidenced by nuclear staining, most prominent in a BRCA1 carrier.

NF-KB p50 Staining in lobules of WT, BRCA1 and BRCA2 carriers



Accomplishments: Figure 5 demonstrate a trend toward increased Ki67 staining in luminal cells from BRCA2 carriers, which is consistent with other assays described above and supports the overall hypothesis of deregulated proliferation in luminal cells of BRCA2 carriers. Figure 6 suggests high NF-KB activity in BRCA1 carriers, which is consistent with prior publications. We show for the first time that BRCA2 carriers do not exhibit this elevated NF-KB signaling, which has important implications for cancer prevention approaches in these patients.

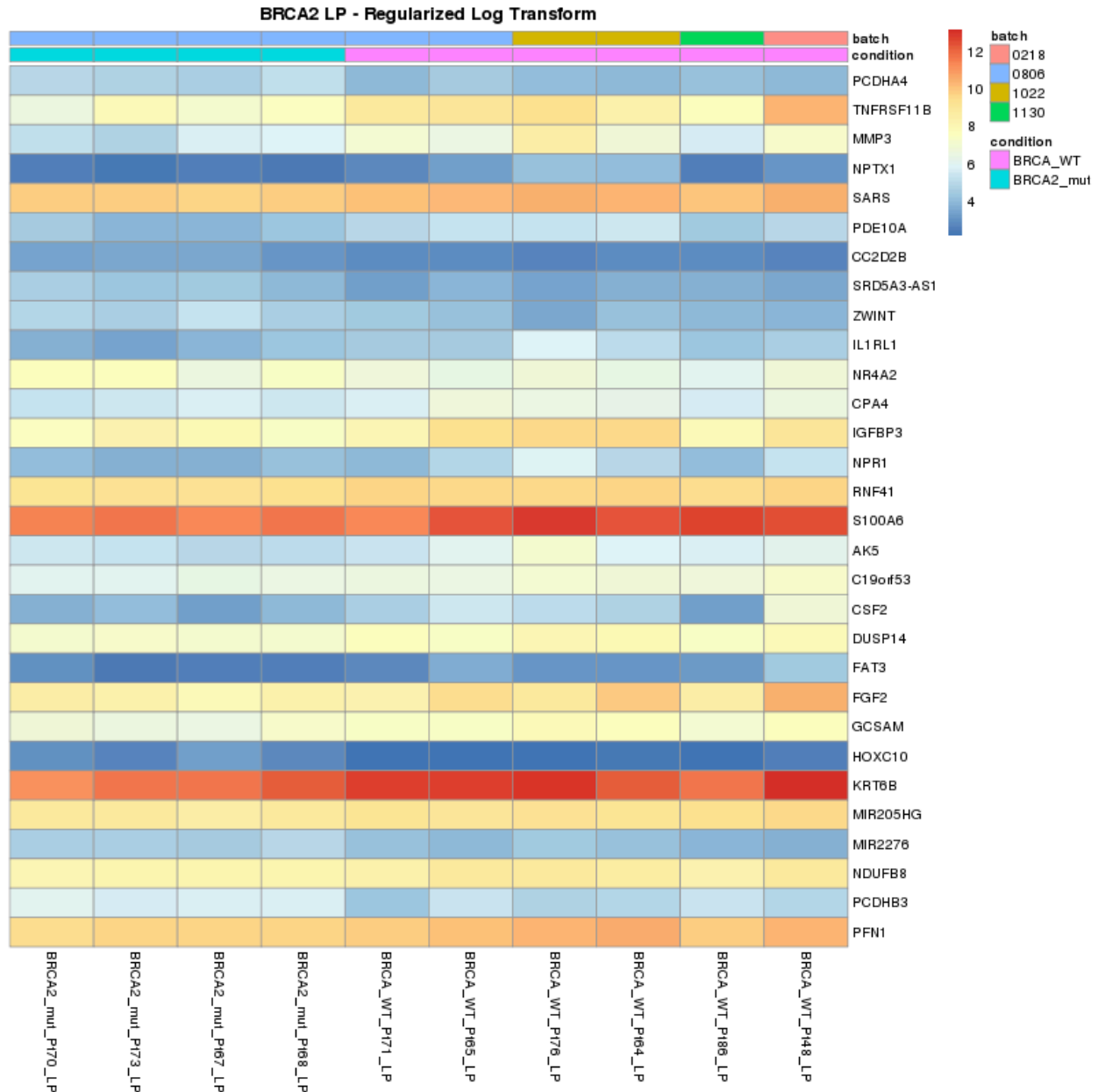
Specific Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.

Major Task 1: Transcriptomic analysis in LP and MASC cells

Methods and accomplishments: (Figure 7 below).

Methods: We carried out RNA sequencing on RNA extracted from primary FACS-sorted mammary epithelial cells from control (N=6), BRCA1 carriers (N=5) and BRCA2 carriers (N=4)

to date. In each case, basal cells, LP cells, mature luminal cells and stromal cells were analyzed separately. Current supervised and unsupervised analyses of this extensive dataset are underway. Our preliminary analysis shown below demonstrates the top differentially expressed genes in LP cells between WT controls (right) and BRCA2 carriers (left). Each column represents one patient; each row represents one gene. Low expression = blue; high expression = red.



Accomplishments: These findings show multiple basal/MASC genes, including MMP3 and IGFBP3, are among the top genes decreased in BRCA2 versus WT LP cells. These findings support the data shown in Figure 4, and demonstrate for the first time decreased basal-like differentiation in LP cells from BRCA2 mutation carriers. Collectively, these findings are consistent with the hypothesis that not only cell numbers but also cell fate is deregulated in the BRCA2 LP population.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Mihriban Karaayvaz, PhD was able to provide training to Devika Salunke, a research assistant in the laboratory who is developing professional skills in preparation for graduate school.

Dr. Karaayvaz herself was able to attend a bioinformatics course, as well as multiple conferences concerning topics related to the area of the proposal.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

1. Presentation at meeting of Consumer Advocates.
2. Presentation in joint laboratory meeting, MGH Center for Cancer Research.
3. Presentation at Scientific Advisory Board Meeting of the Mass General Hospital
4. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat.
5. Presentation at the MGH Cancer Center Annual Retreat.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Plan to complete Aim 2, including genomic analysis, integrated bioinformatic analysis of RNAseq and genomic data, testing of functional pathways and hypotheses gleaned from the bioinformatic analysis.

These studies will set the stage for in vitro and in vivo tests of blocking these pathways in prevention models.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

New types of techniques for human breast tissue analysis and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

None to report at this time.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

None to report to date, but this work is expected to lead directly to application and development of new technology for cancer prevention and clinical trials.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Use of donated tissue proves the value of this approach for scientific advances that benefit patients. This concept will be disseminated through the results of this research.

- 5. CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

None.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

None.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None.

None.

Significant changes in use of biohazards and/or select agents

None.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None to date.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None to date.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Presentation in joint laboratory meeting, MGH Center for Cancer Research.
2. Presentation at Scientific Advisory Board Meeting of the Mass General Hospital
3. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat.
4. Presentation at the MGH Cancer Center Annual Retreat.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None to date.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

New types of techniques for human breast tissue analysis and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None to date.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

New datasets of gene expression in normal and mutated human breast tissue will be a valuable resource and will be publicly available once results of the study are published.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Leif Ellisen
Project Role: PI
Nearest person month worked: 1
Contribution to Project: Oversight of all aspects
Funding Support: This award.

Name: Devika Salunke
Project Role: Research Assistant
Nearest person month worked: 12
Contribution to Project: Conducts all aspects
Funding Support: This award

Name: Kenneth Ross
Project Role: Bioinformatician
Nearest person month worked: 1
Contribution to Project: Bioinformatics
Funding Support: This award

Name: Mihriban Karaayvaz
Project Role: Research Fellow
Nearest person month worked: 6
Contribution to Project: Conducts all aspects
Funding Support: Terri Brodeur Fellowship

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No change in active support for PI/key personnel.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

The project is conducted at the Mass General Hospital.
A portion of cell sorting is conducted at the Ragon Institute of MGH, MIT and Harvard.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award. **N/A**

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments. **N/A**

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. **NONE**